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Escherichia coli O157 Prevalence and Enumeration of Aerobic Bacteria, *Enterobacteriaceae,* and *Escherichia coli* O157 at Various Steps in Commercial Beef Processing Plants†

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ABSTRACT

The effectiveness of current antimicrobial interventions used in reducing the prevalence or load of *Escherichia coli* O157 and indicator organisms on cattle hides and carcasses at two commercial beef processing plants was evaluated. Sponge sampling of beef cattle was performed at five locations from the initial entry of the animals to the slaughter floor to the exit of carcasses from the "hotbox" cooler. For each sample, *E. coli* O157 prevalence was determined and total aerobic bacteria, *Enterobacteriaceae,* and *E. coli* O157 were enumerated. *E. coli* O157 was found on 76% of animal hides coming into the plants, but no carcasses leaving the cooler were identified as contaminated with *E. coli* O157. A positive relationship was seen between the incidence of *E. coli* O157 in hide samples and that in preevisceration samples. Aerobic plate counts and *Enterobacteriaceae* counts averaged 7.8 and 6.2 log CFU/100 cm^2, respectively, on hides, and 1.4 and 0.4 log CFU/100 cm^2, respectively, on chilled carcasses. Aerobic plate counts and *Enterobacteriaceae* counts on preevisceration carcasses were significantly related to the respective levels on the corresponding hides; the carcasses of animals whose hides carried higher numbers of bacteria were more likely to carry higher numbers of bacteria. Implementation of the sampling protocol described here would allow processors to evaluate the efficacy of on-line antimicrobial interventions and allow industrywide benchmarking of hygienic practices.

Escherichia coli O157:H7 has been a pathogen of concern to the meat processing industry for two decades. Cases of hemorrhagic colitis caused by *E. coli* O157:H7 were associated with consumption of undercooked ground beef in the early 1980s *(26).* In the United States during 1992 and 1993, an outbreak of *E. coli* O157:H7 infection associated with consumption of ground beef caused hundreds of illnesses and four deaths *(31).* These events led the Food Safety and Inspection Service (FSIS) to declare the *E. coli* O157:H7 organism an adulterant in ground beef and to require that meat processors establish hazard analysis and critical control point (HACCP) plans for their plants *(12).* Since this time, numerous intervention strategies focusing on prevention of carcass contamination and decontamination of carcasses have been designed, tested, and put into practice at commercial processing plants.

Recent studies have demonstrated that combinations of antimicrobial interventions are more effective at reducing surface contamination on beef tissue than are individual interventions alone *(8, 20, 24).* Many commercial beef processing plants presently employ several interventions (i.e., trimming, steam vacuuming, steam pasteurization, water washes, and organic acid washes) in combination to achieve large reductions in carcass contamination in accordance with their individual HACCP plans *(1).*

In 2002, the FSIS required all raw beef processors to reassess their HACCP plans to ensure that their critical control points were adequately addressing *E. coli* O157:H7 contamination *(13).* In verifying process control, testing for pathogens is generally not useful because of the low numbers of bacterial cells *(6, 17).* Therefore, indicator organisms, present in sufficiently high numbers throughout the processing line, are monitored to ensure that intervention systems are functioning properly. To adequately interpret these data, the relationships between the indicator organisms and the pathogen(s) of interest must be established *(15).*

In this study, counts of indicator organisms (aerobic bacteria and *Enterobacteriaceae*) and *E. coli* O157 and the prevalence of *E. coli* O157 were assessed at various steps in processing to identify relationships that may be exploited to monitor process control. The objectives of this study were twofold: (i) to determine the effectiveness of current interventions used in reducing the prevalence or level of *E.*

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† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

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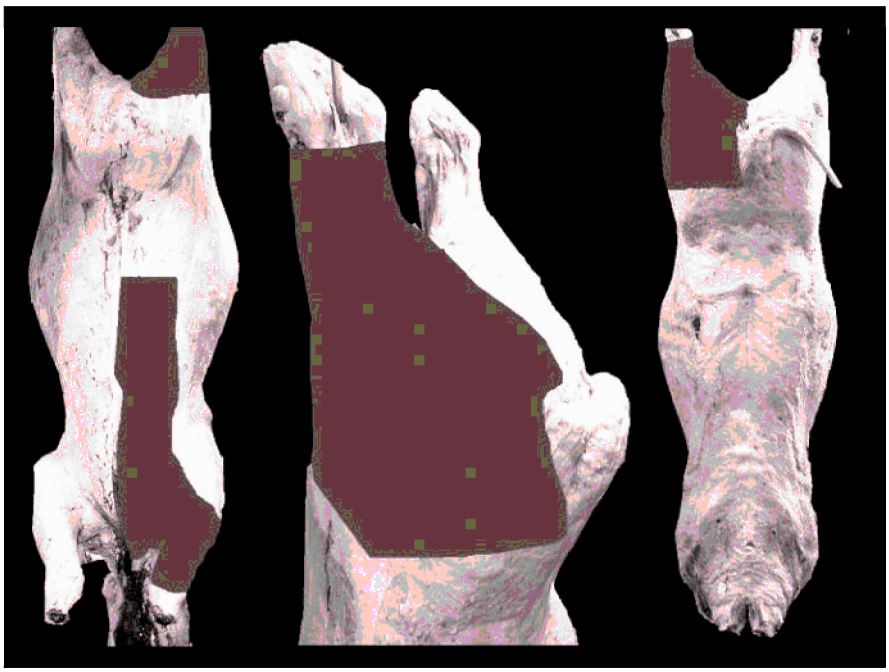


FIGURE 1. *Carcass sampling areas. Shaded areas represent locations where carcass sponge samples were collected.*

coli O157 and indicator organisms and (ii) to develop a tool that can be used by beef processors to monitor their processes.

MATERIALS AND METHODS

Samples were collected during three separate trips each to two commercial fed-beef processing plants. Samples were collected at plant A from the last week in September to mid-October and at plant B from mid-October to the first week of November 2002. Forty-eight samples were collected from each of five sample sites during each trip, for a total of 288 samples per site.

In-plant sampling locations. Sampling with wetted sponges was done at five locations on the processing line: point 1: hide, sampled after hide opening but before hide removal; point 2: preevisceration, immediately following dehiding before any antimicrobial applications; point 3: postevisceration, after evisceration, carcass trimming, and final inspection before the final carcass washes; point 4: postintervention, in the chill cooler after all antimicrobial interventions; and point 5: chilled carcass, 29 h postmortem. Individual animals and carcasses were tagged and tracked throughout the process. The same carcass was sampled at hide (1), preevisceration (2), and postevisceration (3) processing points. The carcass immediately following the carcass sampled at points 1, 2, and 3 was sampled at the postintervention (4) and chilled (5) processing points.

In-plant antimicrobial interventions. The antimicrobial intervention systems utilized by both plants were very similar with regard to the types of interventions employed and their physical placement in the processing line. Prior to sampling point 1, the pattern areas of the hide were subjected to high-pressure water rinses and steam vacuuming. Between sampling points 1 and 2, the hide was removed and the pattern lines were steam vacuumed. Between sampling points 2 and 3, the carcasses were further steam vacuumed, passed through a preevisceration wash cabinet in which they were washed with cold water and 2 to 3% lactic acid, then trimmed and inspected. Between sampling points 3 and 4, the carcasses passed through two wash cabinets, one using 90°C water and the other using peroxyacetic acid, followed by passage through a steam pasteurization cabinet. Sampling point 4 was in the chill cooler, where the carcasses were spray chilled for 29 h before the final samples were taken.

Sample collection. All samples were obtained using Speci-Sponges (Nasco, Fort Atkinson, Wis.) moistened with 20 ml of buffered peptone water (Difco Laboratories, Sparks, Md.). Sponges were wrung out in the bag and then removed from the bag and used to swab the hide or carcass. The hide sample was collected from a 100-cm^2 area over the plate region, using a 100-cm^2 template and flipping the sponge over midway through taking the sample. One sample consisted of approximately five vertical and five horizontal passes (up and down or side to side was considered one pass). For each of the four carcass processing points, two 4,000-cm^2 areas were sampled as a single sample. Sampling of sufficient area at the preevisceration and postevisceration process points was complicated by the carcasses moving at high line speeds. Therefore, efficient sampling was facilitated by using two sponges, each moistened with 20 ml of buffered peptone water. One sponge was used for the inside and outside round area and one for the navel-plate-brisket-foreshank area (Fig. 1). Later, these two sponge samples were combined into one sample bag in the laboratory. For the postintervention and chilled samples, a single sponge was used to sample both areas of the carcass, with one side of the sponge used for the inside and outside round area and the other side of the sponge used for the navel-plate-brisket-foreshank area. All sample bags were transported on ice to the laboratory, where they were processed immediately (plant A samples within 3 h and plant B samples within 6 h). The inside and outside round and navel-plate-brisket-foreshank areas were selected for sampling in this experiment because those areas include or lie adjacent to points where incisions are made to open the hide (hide pattern lines); therefore these points are thought to be hotspots for hide-to-carcass cross contamination. Because contamination is not evenly distributed, large areas were sampled to ensure that the sample was representative at each sampling point.

Sample processing. Sponge bags were massaged thoroughly, and aliquots of 2.5 and 5 ml (12.5% of the diluent volume) were removed from the hide, postintervention, and chilled samples and

TABLE 1. *Percentage of samples positive for* E. coli *O157 for each point by trip and overall*

Sample point[a]	Plant A[b,c]				Plant B[b,c]				Overall
	Trip 1	Trip 2	Trip 3	Total	Trip 1	Trip 2	Trip 3	Total	
Hide									
Mean	83.3	87.5	93.8	88.2 AX	50.0	50.0	89.6	63.2 A	75.7 A
95% CI[d]				81.8–93.0				54.8–63.2	70.3–80.5
Preevisceration									
Mean	25.0	10.4	25.0	20.1 BX	6.5	2.1	18.8	9.2 B	14.7 B
95% CI				13.9–27.6				5.0–15.1	10.8–19.3
Postevisceration									
Mean	6.3	2.1	4.2	4.2 CY	0.0	0.0	10.4	3.5 BC	3.8 C
95% CI				1.5–8.8				1.1–7.9	1.9–6.7
Postintervention									
Mean	0.0	2.1	0.0	0.7 CY	0.0	0.0	0.0	0.0 C	0.3 D
95% CI				0.0–3.8				0.0–2.5	0.0–1.9
Chilled									
Mean	0.0	0.0	0.0	0.0 CY	0.0	0.0	0.0	0.0 C	0.0 D
95% CI				0.0–2.5				0.0–2.5	0.0–1.3

[a] Prevalences within a sample point either (X) differ significantly between plants ($P < 0.01$) or (Y) do not differ between plants ($P > 0.05$).
[b] Percentage of samples positive for *E. coli* O157.
[c] Prevalences in the same column that do not share a common letter (A through E) are significantly different ($P < 0.05$).
[d] CI, confidence interval.

from the preevisceration and postevisceration samples, respectively, prior to the addition of enrichment medium. The sample aliquots were used for enumeration of total aerobic bacteria, *Enterobacteriaceae,* and *E. coli* O157.

Total aerobic plate counts (APC) and *Enterobacteriaceae* counts (EBC) were made on a Bactometer (BioMerieux, Hazelwood, Mo.); for those samples with too few organisms to count on the Bactometer, Petrifilm Aerobic Count Plates or *Enterobacteriaceae* Count Plates (3M Microbiology, St. Paul, Minn.) were used. Bacterial counts from Petrifilm were used to generate standard curve data for the Bactometer during calibration to facilitate data analysis for the two systems.

E. coli O157 cells were counted using a three-tube most-probable-number (MPN) method *(2).* Triplicate 10-fold dilution series were made by transferring 100 μl of the sample aliquot into 900 μl of buffered peptone water plus 50 μg/ml ferrioxamine in deep-well microtrays. The microtrays were incubated at 25°C for 2 h and then at 37°C for 16 to 18 h. After incubation, the trays were kept at 4°C until the results from the *E. coli* O157 prevalence analysis were obtained. For any sample that was positive for *E. coli* O157 in the prevalence analysis, 350 μl from the corresponding buffered peptone water plus ferrioxamine MPN dilutions were subcultured into 4.5 ml of MacConkey broth (Difco). The inoculated MacConkey tubes were incubated static at 42°C for 16 to 24 h. Following incubation, the MacConkey tubes were screened for the presence of *E. coli* O157 using the ImmunoCard Stat! *E. coli* O157 (Meridian Diagnostics, Cincinnati, Ohio). The combination of positive dilution tubes was used to obtain the MPN per ml by using the three-tube MPN table from the *Bacteriological Analytical Manual* (http://vm.cfsan.fda.gov/~ebam/bam-a2.html *(14)).*

***E. coli* O157 detection.** Eighty milliliters of tryptic soy broth was added to the hide, postintervention, and chilled sample bags, and 160 ml of tryptic soy broth was added to the preevisceration and postevisceration sample bags, which contained two sponges. All sample bags were incubated, subjected to immunomagnetic separation, and plated as previously described *(4).* After the plates were incubated, up to three suspect colonies were picked and tested by latex agglutination (DrySpot *E. coli* O157, Oxoid, Basingstoke, UK). In a previous study, Barkocy-Gallagher et al. *(2)* demonstrated that >90% of samples that were presumptively positive for *E. coli* O157 based on the above methods were actually positive for *E. coli* O157:H7. Therefore, for the purposes of this study, any sample that produced characteristic *E. coli* O157 colonies that gave positive reactions for the O157 latex agglutination assay was considered positive for *E. coli* O157:H7.

Statistical analysis. To compare the prevalence of *E. coli* O157 between plants and among sampling sites, continuity-adjusted chi-square *P*-values were calculated using the PROC FREQ procedure of SAS *(27).* When multiple comparisons were made, the pair-wise *P*-values were adjusted using Hommel's modification of the Bonferroni procedure *(16)* to avoid inflated type I error rates. APC and EBC data were log transformed before analysis of variance (ANOVA). For each sampling site, a one-way ANOVA was conducted to determine whether counts differed between plants. For each plant and for the combined data set, a one-way ANOVA was conducted to determine whether counts differed among sampling sites. Hide and preevisceration observations were grouped on the basis of APC or EBC, and chi-square analysis was conducted to determine whether there was a relationship between APC or EBC and the prevalence of *E. coli* O157:H7.

RESULTS AND DISCUSSION

***E. coli* O157 prevalence and enumeration.** *E. coli* O157 contamination was found on 75.7% (218 of 288) of the cattle hides tested, with a range of 50 to 93.8% among the six sampling trips (Table 1). On average, the hides of

TABLE 2. E. coli *O157 MPN results*

Sample point	MPN[a]	Plant A[b]	Plant B[b]
Hide	ND[c]	18	53
	<60	54	67
	60–99	25	14
	100–999	28	5
	1,000–9,999	12	5
	10,000–99,999	6	0
	100,000–999,999	1	0
Preevisceration	ND	115	131
	<1.5	24	11
	1.5–9	4	2
	10–99	0	0
	100–999	1	0

[a] Log CFU/100 cm^2.
[b] Number of samples for each class of MPN values.
[c] ND, MPN not done because O157 was not detected by culture method.

cattle processed at plant A carried *E. coli* O157 more frequently ($P < 0.0001$) than did those of cattle processed at plant B (88.2% versus 63.2%), although the final sampling period at plant B had the second highest *E. coli* O157 hide prevalence of the six sampling trips.

Immediately following hide removal, the mean *E. coli* O157 prevalence on carcasses was 14.7% (range of 2.1 to 25%) for the various sampling trips. Carcasses sampled prior to evisceration at plant A were twice as likely ($P < 0.0001$) to have detectable *E. coli* O157 contamination as were those at plant B (20.1% versus 9.2%).

Following preevisceration sampling, the carcasses were washed with a lactic acid solution, eviscerated, split, trimmed, inspected according to governmental regulations, and then sampled again. The mean *E. coli* O157 prevalence at the postevisceration sampling point had decreased to 3.8% (range of 0.0 to 10.4%) among the six sampling trips, and there was no significant difference ($P > 0.05$) between plants.

E. coli O157 was detected on only 1 of 288 (0.3%) carcasses sampled after the application of all antimicrobial interventions. None of the chilled carcass samples were positive for *E. coli* O157.

The prevalence of *E. coli* O157 described herein is analogous to that reported for beef carcasses sampled in the fall of 2001 *(2)*. In that report, the hide, preevisceration, and postintervention prevalence levels of *E. coli* O157:H7 were 67.2, 27.3, and 1.0%, respectively. Sampling and detection methodologies used in that study were similar to those used here.

***E. coli* O157 enumeration.** Consistent with the *E. coli* O157 prevalence data, MPN levels generally were higher for plant A than for plant B (Table 2). Enumeration of *E. coli* O157 cells revealed that although most *E. coli* O157-positive hides (56%) were contaminated at low levels, with O157 levels below the MPN detection limit (<60 MPN/100 cm^2), many hides (41%) had levels between 60 and 9,999 MPN/100 cm^2 and a few (3%) had dense populations of *E. coli* O157 (one sample had >220,000 MPN/100 cm^2). As a whole, the *E. coli* O157 MPN levels may be underestimated. Certain MPN combinations, specifically those indicative of growth suppression at low dilutions, appear more often than would be expected by chance. This phenomenon has been seen previously when examining bacteria in various food matrices *(5, 14)*. Lahti et al. *(21)* used an MPN method to enumerate *E. coli* O157 in fecal samples from bulls and documented a large range (<0.2 to >160,000 MPN/g of feces) in the *E. coli* O157 populations associated with individual animals.

The numbers of *E. coli* O157 organisms present on most of the positive carcasses (83%) were below the detection limit of the MPN method used (<1.5 MPN/100 cm^2), but six carcasses had O157 loads of approximately 2 MPN/100 cm^2 and one carcass had an O157 load of 550 MPN/100 cm^2. Barkocy-Gallagher et al. *(2)* reported similar *E. coli* O157 MPN levels from preevisceration samples collected in the fall of 2001. The *E. coli* O157 populations for 69% of the positive carcass samples from that study were below the MPN detection limit, but one carcass carried an *E. coli* O157 load of 334 MPN/100 cm^2. All of the postevisceration and postintervention carcasses that harbored *E. coli* O157 did so at levels below the detection limit of the MPN assay used here (<1.5 MPN/100 cm^2, data not shown).

Enumeration of aerobic bacteria and *Enterobacteriaceae*. Other researchers have stated that bacterial counts, such as APC and EBC, are not correlated with levels of pathogens and cannot be used with index organisms to gauge the presence or absence of specific pathogens *(15, 19)*. Instead, the bacteria enumerated in these tests have been used as indicator organisms to assess the hygienic status of processing plants and the performance of antimicrobial interventions *(15)*. *Enterobacteriaceae* have been considered suitable indicators for the effectiveness of decontamination procedures because they are slightly more tolerant to lactic acid than are several enteric pathogens *(29)*. If the indicator organisms react to antimicrobial interventions in a manner similar to that of the pathogen of interest, in this case *E. coli* O157:H7, then these organisms can be used to monitor process control.

The indicator organism counts for hides were higher for plant A than for plant B (Table 3), similar to the trend for *E. coli* O157. The average values for APC on cattle hides ranged from 8.0 to 9.0 log CFU/100 cm^2 for plant A and 7.2 to 7.4 log CFU/100 cm^2 for plant B, and the EBC trip averages ranged from 6.7 to 8.0 log CFU/100 cm^2 for plant A and 4.9 to 5.8 log CFU/100 cm^2 for plant B.

During hide removal, bacterial deposition onto the sterile surface of the carcass resulted in levels of 3.5 and 1.4 log CFU/100 cm^2 for APC and EBC, respectively. Given the reduction seen in *E. coli* O157 prevalence between the preevisceration and postevisceration sampling points, we assumed that the corresponding EBC and APC levels also would decrease. This was not the case; the APC and EBC results showed a slight increase, resulting in postevisceration levels of 3.7 and 1.7 log CFU/100 cm^2, respectively.

TABLE 3. *Mean APC and EBC for each sampling point by trip and overall*

Indicator organism	Sample point	Plant A[a,b]				Plant B[a,b]				Overall
		Trip 1	Trip 2	Trip 3	Total	Trip 1	Trip 2	Trip 3	Total	
APC[c]	Hide	9.0	8.1	8	8.3 AX	7.2	7.3	7.4	7.3 A	7.8 A
	Preevisceration	4.2	3.6	3.7	3.8 BX	3.0	3.2	3.3	3.2 B	3.5 C
	Postevisceration	3.8	3.4	3.9	3.7 CY	3.3	4.1	4.0	3.8 C	3.7 B
	Postintervention	1.4	1.4	1.0	1.2 EY	0.9	1.4	1.5	1.3 D	1.3 E
	Chilled	1.6	0.9	1.8	1.4 DY	2.0	1.1	1.1	1.4 D	1.4 D
EBC[c]	Hide	8.0	6.6	6.7	7.1 AX	5.5	4.9	5.8	5.4 A	6.2 A
	Preevisceration	2.5	1.4	1.4	1.8 BX	1.2	0.7	1.0	1.0 C	1.4 C
	Postevisceration	1.9	1.3	1.3	1.5 CX	1.8	1.7	2.1	1.9 B	1.7 B
	Postintervention	0.4	0.1	0.0	0.2 DY	0.1	0.1	0.2	0.1 E	0.2 E
	Chilled	0.3	0.2	0.6	0.3 DY	0.7	0.5	0.3	0.5 D	0.4 D

[a] Log CFU/100 cm^2.
[b] Means in the same column for a bacterial type that do not share a common letter (A through E) are different ($P < 0.05$).
[c] Means within a sample site either (X) differ between plants ($P < 0.0001$) or (Y) do not differ between plants ($P > 0.05$).

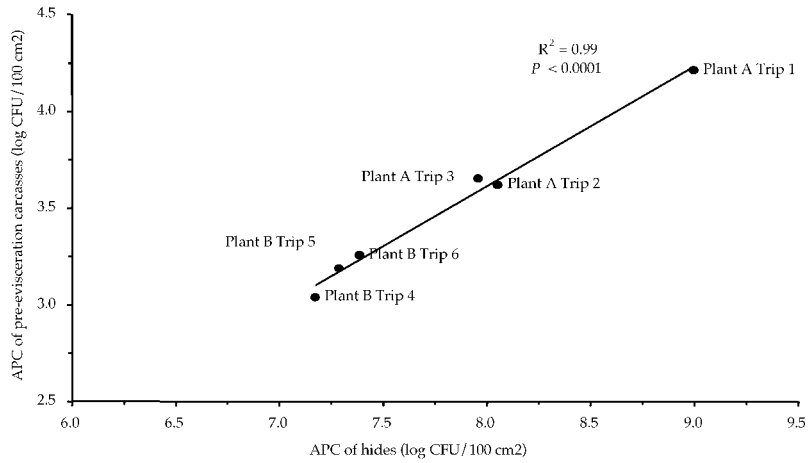
There are numerous possible explanations for this discrepancy. It is possible that the preevisceration lactic acid rinse had a greater effect on *E. coli* O157:H7 than it had on the indicator organism population. In vitro studies have shown that gram-negative bacteria are more susceptible to lactic acid than are gram-positive bacteria *(32).* This fact could explain the lack of reduction seen in the APC. However, based on the results from 20 years of research, Smulders and Greer *(30)* stated that one should expect an average reduction of 1.5 log in APC from lactic acid treatment of carcasses. Alternatively, based on previous in-plant reports indicating that preevisceration lactic acid rinses result in a reduction of APC, coliforms, and generic *E. coli (9, 10),* a second scenario is also possible. The total bacterial population could have been reduced by the preevisceration lactic acid rinse, and carcasses could have become subsequently recontaminated with bacteria other than *E. coli* O157:H7 during evisceration, splitting, trimming, and/or inspection. These activities may have led to the deposition of additional bacteria onto the carcass, masking any reduction due to the organic acid wash. We are aware of only one other study in which carcasses in commercial facilities were sampled both before preevisceration rinsing and at a similar postevisceration point (before final interventions) *(11).* In that study, the prevalence of *E. coli* O157:H7 decreased between these points, as was the case in the present study, but levels of other bacteria were not studied. Further studies are needed to identify which scenario is correct.

Following postevisceration sampling, the carcasses were subjected to washes with hot water and peroxyacetic acid and to steam pasteurization. These interventions reduced APC and EBC to 1.3 and 0.2 log CFU/100 cm^2, respectively. Cooling the carcasses via spray chilling and storage at refrigerated temperatures held the bacterial populations essentially constant with minimal growth, leading to chilled sample numbers of 1.4 and 0.4 log CFU/100 cm^2 for APC and EBC, respectively.

Relationships between hide and carcass contamination. Levels of APC, EBC, and to a lesser extent *E. coli* O157 prevalence on hides were significantly related to the respective levels on the corresponding carcasses (Fig. 2). When grouped by sampling trip, groups with larger bacterial loads and higher incidence rates on the hide subsequently had larger bacterial loads or higher incidence rates on the carcass. This association was stronger for aerobic bacteria and *Enterobacteriaceae* than for *E. coli* O157. In previous studies, antimicrobial interventions focused on the hide, such as dehairing, were translated into lower overall bacterial loads and lower incidences of pathogens on carcasses *(7, 23).* Nou et al. *(23)* demonstrated that hide dehairing, which in effect sanitizes the animal hide, can greatly reduce carcass bacterial loads and eliminate *E. coli* O157. Barkocy-Gallagher et al. *(3)* showed that >66% of the *E. coli* O157:H7 isolates from beef carcasses identified late in processing could be tracked by pulsed-field gel electrophoresis to the same carcass early in processing. This finding indicates that the *E. coli* O157 population associated with the animal or carcass persisted throughout processing. These facts indicate that interventions focused on reducing the number of bacteria and pathogens on the hide can have a large impact on the levels of pathogens on the carcass.

Relationships between the levels of APC and EBC and the incidence of *E. coli* O157 on carcasses. When the APC and EBC levels were grouped into classes, significant associations were seen between the APC or EBC class and the prevalence of *E. coli* O157 for carcass samples ($P < 0.05$). The samples from higher APC and EBC classes were more likely to be positive for *E. coli* O157 (Table 4). Caution should be taken when interpreting these results because the data set was limited to 288 samples from only two different plants in one season. This relationship may be the result primarily of differences among trips or between plants rather than a direct relationship between APC and EBC and prevalence of *E. coli* O157. Therefore, more data are needed to confirm these findings. A similar analysis was done by Siragusa et al. *(28),* where APC values from preevisceration, postintervention, and chilled samples were grouped into classes and relationships with the incidence of *E. coli* biotype 1, an indicator of fecal contamination, were assessed. In that study, a strong association

A.



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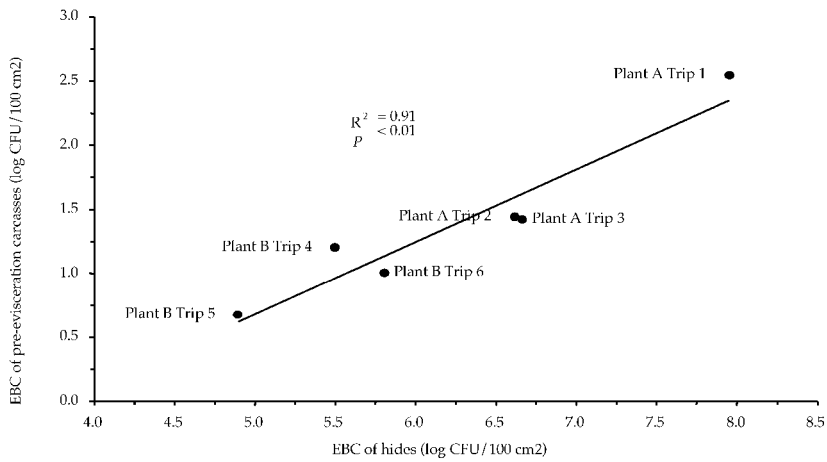

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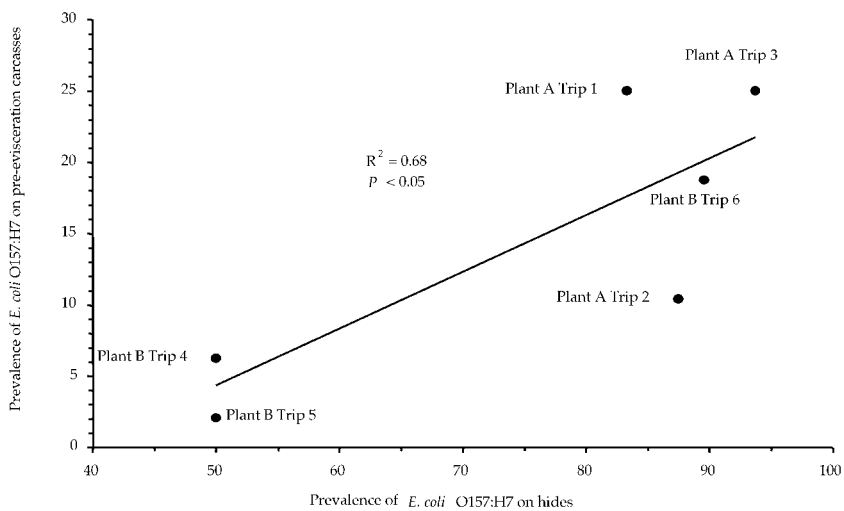

FIGURE 2. *Correlations between hide and preevisceration bacterial levels. (A) APC levels for the preevisceration and hide samples are plotted for each sampling trip* (n = *48 per trip). (B) EBC levels for the preevisceration and hide samples are plotted for each sampling trip* (n = *48 per trip). (C)* E. coli *O157 prevalence for the preevisceration and hide samples is plotted for each sampling trip* (n = *48 per trip).*

was identified between APC class and the incidence of an *E. coli*–positive sample for the overall data set. However, no such relationship was seen when the analysis was restricted to the preevisceration samples. Although indicator organism levels cannot be used for direct presence or absence analysis of *E. coli* O157, they may be useful as a guideline for the minimization of *E. coli* O157 contamination. By modifying intervention schemes to maintain APC and EBC levels below maximum target values, processors are likely to reduce the prevalence of *E. coli* O157 on carcasses.

Efficacy of interventions. Both plants employed the concept of multiple sequential interventions using very

TABLE 4. *Relationship between APC and EBC of preevisceration carcasses and prevalence of* E. coli *O157 on preevisceration carcasses*

Indicator organism	Log CFU/100 cm^2	*n*	No. (%) of *E. coli* O157–positive samples[a]
APC	>4	59	14 (24) A
	<4	227	28 (12) B
EBC	>2	57	14 (25) A
	<2	228	27 (12) B

[a] Percentages within an indicator organism that do not share a common letter (A, B) are significantly different ($P < 0.05$).

similar antimicrobial applications. By minimizing deposition of bacteria onto the carcass and using subsequent effective decontamination, the processors were able to maintain *E. coli* O157 populations at less than detectable levels on all of the carcasses tested after chilling.

HACCP testing. In light of the requirement that beef processing plants reassess their HAACP plans regarding the efficacy of the antimicrobial interventions used to control *E. coli* O157:H7, we offer the sampling method detailed here for use by the industry as a tool to evaluate process control and validate critical control points. This method offers clear advantages over those previously reported for HAACP monitoring *(18).* First, the technique is noninvasive and does not result in any product loss. Second, the procedure can be performed efficiently and adequately on the line at operational chain speeds. Third, the microbiologic hygiene of a plant is assessed at points encompassing the carcasses' entrance to and exit from the slaughter floor. Several researchers have demonstrated that the hide is possibly the major source of beef carcass contamination *(2, 22, 23, 25).* Therefore, processors must know the bacterial levels on animals coming into the plants to accurately evaluate antimicrobial intervention performance at various points in the processing line. An industrywide standard protocol for microbiologic testing would allow individual plants to benchmark their hygienic standard and evaluate the efficacy of current on-line antimicrobial interventions.

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